REMARKS

Claims 31-35, 37-46 and 48-54 remain pending. Favorable reconsideration is respectfully requested.

Applicants would like to thank Examiner Mohamed for the helpful and courteous discussion held with their representative on May 18, 2004. During that discussion, Applicants' representative pointed out that Kitamura et al. describe complex formation with iodine, and not to refolding proteins. Applicants' representative also pointed out that the cyclodextrins described by Larsen et al. are outside the scope of the claims, and that this reference also fails to describe refolding proteins. The following remarks expand on the discussion with the Examiner.

The present invention relates to a kit for refolding denatured protein, comprising (a) a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150 and (b) a polyoxyethylenic detergent. See Claim 31.

The present invention also relates to a kit for refolding denatured protein, comprising (a) a cyclic saccharide cycloamylose having a polymerization degree of from 25 to 150 and (b) an ionic detergent. See Claim 35.

The present invention also relates to a method of refolding a denatured protein, comprising:

contacting a polyoxyethylenic detergent with a denatured protein, followed by

contacting the protein with a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150, to produce a folded protein. See Claim 39.

The present invention also relates to a method of refolding a denatured protein, comprising:

contacting an ionic detergent with a denatured protein, followed by

contacting the protein with a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150, to produce a folded protein. See Claim 46.

The rejection of the claims under 35 U.S.C. §103(a) over Daugherty et al. taken with Kitamura et al. and Larsen et al. is respectfully traversed. The cited references fail to suggest the claimed kits and methods of refolding a denatured protein.

The present invention is based on the discovery that the very large cyclic saccharide cycloamylose recited in the pending claims, i.e., having a degree of polymerization of 25 to 150, in combination with a polyoxyethylenic detergent (see independent Claims 31 and 39) or an ionic detergent (see independent Claims 35 and 46), overcomes the problems associated with β-cyclodextrin (β-CD).

As described in the specification at the bottom of page 3, the β-cyclodextrin used by Daugherty et al. has problems associated with stability, and is not completely satisfactory. This is a critical difference between the present invention and Daugherty et al.: the cycloamylose used in the present invention has a much, much higher degree of polymerization, i.e., the number dextrin moieties.

There is no prima facie case of obviousness with respect to Daugherty et al. in view of Kitamura et al. and Larsen et al.

Daugherty et al. describe protein refolding using β-cyclodextrin. See the Table at page 33963 of the reference. In fact, β-cyclodextrin is the only cyclic saccharide described in that reference. The β-cyclodextrin used in Daugherty et al. has a polymerization degree of 7 (See Machida et al., FEBS Letters, 486, (2000), pp. 131-135, of record, at page 131, second column, second full paragraph, lines 1-3). Therefore, Daugherty et al. fail to describe a cyclic saccharide cycloamylose having a polymerization degree of from 25-150 in combination with

the claimed detergents as a protein refolding agent. In addition, Daugherty et al. fails to even suggest that other cyclodextrins can be used to refold proteins.

In fact, Daugherty et al. describe that with regard to the ability of CD to remove detergent, the cavity of α-CD is too small to bind to detergent like Triton X-100, but β-CD can bind tightly without such a problem (see page 33966, right column, 13 to 6 lines from the bottom).

Kitamura et al. disclose complex formation between cyclodextrins (cycloamylose) having a degree of polymerization of 21-32 and iodine, i.e., I_2. See the Abstract.

In the Introduction Section of the reference, Kitamura et al. state the large cyclodextrins having a degree of polymerization of from 17 to several hundred are known (see the first paragraph in column 1 at page 612), and also state that:

> These large CDs [cyclodextrins] may have the potential to function as host molecules for a variety of organic reagents and iodine which is ***different from the common cyclodextrins, since it is likely that the large CDs have a cavity geometry which is different from that of smaller CDs***. [Emphasis added.]

Kitamura et al. demonstrate that the large CDs are indeed very different from the smaller CDs. As described in the Results and Discussion section beginning in the second column at page 612 of the reference, Kitamura et al. explain that the complex formed with the small cyclodextrin CD6 formed a 1:1 complex with iodine. In contrast, the data obtained for the much larger cyclodextrin CD26 indicated the formation of a 1:2 complex in which two identical binding sites in the cyclodextrin each bound an iodine molecule. In the paragraph bridging pages 613-614 of the reference, Kitamura et al. explain that the thermodynamic data obtained for a CD having a degree of polymerization of 120 (CD120) were similar to those of extended linear amylase. In fact, Kitamura et al. state:

> Thus, if the DP is sufficient to allow the molecule to behave as flexible chain [sic], the local conformation may be similar to that of a long linear amylase chain.

In addition, Kitamura et al. are completely silent with respect to refolding denatured proteins. There is no mention at all of protein folding in that reference.

Larsen et al. disclose the formation of complexes between cyclodextrins having a degree of polymerization of 6 to 17 and a variety of anions. See the Abstract and column 2 at page 153 of the reference. The results are shown in Table 1 at the top of page 155.

This reference fails to describe cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150 as recited in the claimed kits and methods. The cyclodextrins used by Larsen et al. are outside of the claimed range (too small). In addition, those cyclodextrins are smaller as compared to the CD26 and CD126 cyclodextrins reported in Kitamura et al.

The reference reports that the data is consistent with predominant 1:1 complex formation between the cyclodextrins and the anions. See the Results and Discussion paragraph at page 154, column 2 of the reference. Larsen et al. also report that "the larger CD showed overall lower binding capacities" as compared to β-CD. See the Results and Discussion paragraph at page 154, column 1 of the reference. In the paragraph bridging columns 1 and 2 at page 156 of the reference, Larsen et al. state:

> The results strongly suggest that the cavities of the large CD are much smaller than would be expected if they had a planar structure like α-, β-, and γ-CD.

Thus, Larsen et al. report that the structure of β-CD and the CDs having degree of polymerization of 9 to 17 have different structure.

Larsen et al. are also completely silent with respect to refolding denatured proteins. There is no mention at all of protein folding in that reference.

There is simply no suggestion in these references to substitute the cycloamylose described in Kitamura et al. for the β-cyclodextrin described in Daugherty et al. Daugherty et al. do not describe any shortcomings of β-cyclodextrin which would motivate one to use the large-ring cyclodextrins described in Kitamura et al. As discussed above, Kitamura et al. describe complex formation between cyclodextrins (cycloamylose) having a degree of polymerization of 21-32 and iodine, i.e., I2, and make no mention of using cyclodextrins to refold denatured proteins. The cyclodextrins described in Larsen et al. do not even overlap those specified in the claimed kits and methods.

Based on the comments in the Official Action dated April 20, 2004, the logic of the rejection appears to be that since Kitamura et al. and Larsen et al. describe that CDs larger than β-CD can form inclusion complexes, then those references provide a reasonable expectation that the larger CDs can be used to successfully refold proteins.

However, as discussed above, that logic ignores the fact that the combined disclosure of Larsen et al. and Kitamura et al. suggest that the CDs recited in the claimed kits and methods have different properties as compared to β-CD.

Larsen et al. report that β-CD and the larger CDs used in that reference, i.e., degree of polymerization of 9 to 17, formed a 1:1 complex with the anions. However, Kitamura et al. disclose that even larger CDs having a degree of polymerization of 21 to 32 form a 1:2 complex with iodine.

In addition, Larsen et al. state that the results obtained in their study suggest that the larger CDs do not have a planar structure like β-CD because the cavities of those larger CDs are smaller than what would be expected if they had a planar structure. In fact, Kitamura et al. report that a CD having a degree of polymerization of 120 behaves as a flexible chain in which "the local conformation may be similar to that of a long linear amylase chain."

Also, Larsen et al. presents the result that β-CD was the best complex-forming compound as compared to α-CD and γ-CD (see page 155, left column, lines 6-9). Kitamura et al. demonstrate that CDs having a degree of polymerization (DP) of 21 to 33 is quite different from β-CD in terms of thermodynamic value such as binding constants with respect to complex formation with iodine in aqueous solution (see page 614, Figs. 3 and 4). In addition, many publications have described an obvious and well-known fact that β-CD has a greater ability to form inclusion compounds as compared to other CDs.

It is considered that the reason why Daugherty et al. used β-CD is a result of considering this fact, and it is clear that there is no motivation to substitute other CDs for β-CD of Daugherty et al. Therefore, for one skilled in the art, it is difficult to combine Larsen et al. or Kitamura et al. with Daugherty et al.

Larsen et al. describes that β-CD was found to be the best complex-forming compound followed by α- and γ-CD (see page 156, Table 2), and further, CD of 9 to 15 showed overall lower binding capacities (page 155, left column, lines 15-25).

Moreover, Kitamura et al. describes that CD having a DP large enough to behave as a flexible chain may have structure similar to that of linear amylase (see page 613, right column, penultimate line to page 614, left column, lines 5-9). Those descriptions in Larsen et al. and in Kitamura et al. could reflect the intention that CDs having a DP larger than β-CD do not have practicality superior to that of β-CD, which those skilled in the art would have had at the time of filing of the present application.

During the personal discussion held on May 18, 2004, the Examiner informed Applicants' representative that a major underpinning of the rejection is the first paragraph in column 1 at page 612 of Kitamura et al. In fact, when Applicants' representative asked where the secondary references provide a reasonable expectation that a cyclic saccharide

cycloamylose having a polymerization degree of from 25-150 in combination with the recited detergents could be used successfully as a protein refolding agent, the Examiner referred to that paragraph.

The first sentence of the paragraph states that the "common cyclodextrins (α-, β-, and γ-CD) form inclusion compounds with small molecules." The second sentence cites Takaha et al. (of record) for the fact that larger CDs having a degree of polymerization are known. The next sentence was quoted above in the discussion of the Kitamura et al. reference, and is a speculation by those authors that the larger CDs may function as hosts "in a manner which is different from the common cyclodextrins, since it is likely that the large CDs have a cavity geometry which is different from that of smaller CDs." The next several sentences discuss crystal structures and inclusion studies of larger CDs. The last three sentences in that paragraph summarize the purpose of the study described in the reference itself.

In view of the disclosure of the paragraph discussed above, that section of Kitamura et al. certainly fails to suggest substituting the β-CD used by Dougherty et al. with the larger CDs studied by Kitamura et al.

Based on the foregoing, the claimed kits and methods are not prima facie obvious over the combination of Daugherty et al., Kitamura et al., and Larsen et al.

In addition, the experimental data set forth in the present specification provides striking evidence of non-obviousness. Table 1 of the present application shows the experimental result of refolding a protein, where Tween 40 was used as a detergent in combination with various cyclic saccharides. In comparing the refolding effect on denatured by each combination, cyclic saccharide cycloamylose CA(S) and CA(L) having a polymerization degree of 25 to 150 recovered 140% and 120% of activity, respectively. β-cyclodextrin of polymerization degree of 7 recovered 120% of activity as well, but this

substance has a defect as described above. The result of γ-cyclodextrin having a degree of polymerization 8 was 8% of the recovery and no refolding effect was observed. A similar experiment was carried out with a cyclic saccharide having a polymerization degree of 10 to 14, resulting in a recovery of only 4% of activity. Therefore, all the cyclic saccharides having various polymerization degrees do not always show a refolding effect. It is not expected from the cited references that the cyclic saccharide cycloamylose of the present invention, which has a much higher polymerization degree than the β-cyclodextrin of polymerization degree of 7, yields a far superior refolding effect.

In the Example 1 of the present application, citrate synthase (CS) is first denatured with guanidine hydrochloride and then refolded with a variety of artificial chaperones. See page 10 and page 13 of the specification. The results are presented in Table 1 at page 18 of the present specification.

The results of this experiment are set forth at page 19, first two paragraphs, of the specification which read as follows:

> Moreover, as to the change with the passage of time of the enzymatic activity, as apparent from Figs. 1 and 2, it has become clear that, in case of the artificial chaperon using CA(S) and CA(L) as the cyclic saccharide, the enzyme was refolded into the active form within as short as 2 hours after the addition of cycloamylose. That is, this shows that the artificial chaperon of the present invention has the ability of refolding the denatured protein in an unfolded state correctly within a short time.
>
> On the other hand, in case of β-CD, only from about 30 to 40% of the enzymatic activity was recovered 2 hours after the addition of the β-CD, and it took more than overnight to recover 100% of the enzymatic activity.
>
> Therefore, it has become clear that cycloamylose is more preferable agent used as the artificial chaperon of the present invention.

As described at page 17 of the specification, Figure 1 of the present application presents the time course of the recovery of enzymatic activity using different cyclic saccharides and Tween 40, and Figure 2 presents similar data for Tween 60. As stated in the passage from the specification described above, using the claimed re-folding agent the enzyme was refolded into the native form within as short a time period as 2 hours. In contrast, with β-cyclodextrin, only about 30 to 40% of the enzymatic activity was recovered in 2 hours, and it took more than an overnight incubation to recover 100% of the enzymatic activity.

The cited references fail to suggest these striking results.

Daugherty et al. describe protein refolding using β-cyclodextrin. This reference fails to describe the cyclic saccharide cycloamylose having a polymerization degree of from 25-150 in combination with the specified detergents as claimed.

Kitamura et al. disclose complex formation between cyclodextrins (cycloamylose) having a degree of polymerization of 21-32 and iodine, i.e., I2. That reference fails to even mention protein refolding.

Larsen et al. describe the formation of complexes between cyclodextrins having a degree of polymerization of 6 to 17 and a variety of anions. That range is outside the degree of polymerization specified in the claimed kits and methods. In addition, Larsen et al. are also completely silent with respect to refolding denatured proteins. There is no mention at all of protein folding in that reference.

One with Daugherty et al., Kitamura et al., and Larsen et al. in hand would not have predicted that a cyclic saccharide cycloamylose having a polymerization degree of from 25-150 would be dramatically more effective as compared to β-cyclodextrin for refolding proteins as demonstrated by the data presented in the present specification. Daugherty et al. describe protein refolding using β-cyclodextrin and is silent with respect to a cyclic

saccharide cycloamylose having a polymerization degree of from 25-150. Neither Kitamura et al. nor Larsen et al. even mention refolding proteins. Given the teachings of the references, one would simply not be led to expect the striking results set forth in the present specification.

In addition, Example 1 of the present application was repeated using, as CD, CD mixture having DP shown in the Table 1 below and using Tween 40 as a detergent. The result of assay of enzymatic activity of CS is shown in Table 1.

Table 1

DP of CD	6	7	8	10	14	25-50	40-150
Tween 40	50	120	8	4	2	140	120

Table 1 clearly indicates that the enzymatic activity observed in CD of DP 6 or 7 was barely observed in CD of 8, 10 and 14, but surprisingly, both CD of DP 25 to 50 and CD of DP 40 to 150 show an enzymatic activity which is the same as or even higher than CD of DP 7 (i.e., β-CD). This result is opposite to what one have predicted at the time of filing the present application, i.e., the expectation would have been that CD having larger DP than that of β-CD does not have practicality superior to that of β-CD. Or in other words, the result is an unexpected finding. Considering the common knowledge of those skilled in the field as represented by the result of CD of DP up to 15 shown in Larsen et al., it would not have been expected that a CD of DP 25 or more would show such an excellent effect.

In the Advisory Action dated February 14, 2003, the Examiner criticized the experimental data discussed above on the grounds that "there is nothing in the claims to tie decreased refolding time and stability of the protein to the components of the kit and methods of refolding denatured protein." In response, Applicants note that the decreased refolding time and stability of the protein are inherent properties of the claimed kit and the use of the

components of the kit in the claimed method. The purpose of the claims is to define the invention. The results obtained with the kit, i.e., decreased refolding time and stability of the protein, are described in detail in the specification of the present application, and the patentability of the pending claims does not require explicit recitation of those properties in the claims.

Based on the foregoing, the combination of Daugherty et al., Kitamura et al., and Larsen et al. fail to suggest the claimed kits and methods. In addition, the striking experimental data set forth in the specification would not have been predicted from those references. Therefore, the claimed kits and methods are not obvious over those references. Accordingly, withdrawal of this ground of rejection is respectfully requested.

Applicants submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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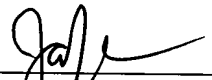

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APPENDIX

The pending claims read as follows:

31. A kit for refolding denatured protein, comprising (a) a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150 and (b) a polyoxyethylenic detergent.

32. The kit of Claim 31, wherein the polyoxyethylenic detergent is selected from the group consisting of polyoxyethylenesorbitan ester, polyoxyethylenedodecyl ether, polyoxyethyleneheptamethylhexyl ether, polyoxyethyleneisooctylphenyl ether, polyoxyethylenenonylphenyl ether, polyoxyethylene fatty acid ester and sucrose fatty acid ester.

33. The kit of Claim 31, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50.

34. The kit of Claim 31, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150.

35. A kit for refolding denatured protein, comprising (a) a cyclic saccharide cycloamylose having a polymerization degree of from 25 to 150 and (b) an ionic detergent.

37. The kit of Claim 35, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50.

38. The kit of Claim 35, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150.

39. A method of refolding a denatured protein, comprising:

contacting a polyoxyethylenic detergent with a denatured protein, followed by

contacting the protein with a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150, to produce a folded protein.

40. The method of Claim 39, wherein the polyoxyethylenic detergent is selected from the group consisting of polyoxyethylenesorbitan ester, polyoxyethylenedodecyl ether, polyoxyetheyleneheptamethylhexyl ether, polyoxyethyleneisooctylphenyl ether, polyoxyethylenenonylphenyl ether, polyoxyethylene fatty acid ester and sucrose fatty acid ester.

41. The method of Claim 39, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50.

42. The method of Claim 39, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150.

43. The method of Claim 39, wherein the folded protein has an α-helical structure.

44. The method of Claim 39, wherein the folded protein has an β-sheet structure.

45. The method of Claim 39, wherein the refolded protein has an intramolecular S-S bond.

46. A method of refolding a denatured protein, comprising:

contacting an ionic detergent with a denatured protein, followed by

contacting the protein with a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150, to produce a folded protein.

48. The method of Claim 46, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50.

49. The method of Claim 46, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150.

50. The method of Claim 46, wherein the folded protein has an α-helical structure.

51. The method of Claim 46, wherein the folded protein has an β-sheet structure.

52. The method of Claim 46, wherein the refolded protein has an intramolecular S-S bond.

53. The kit of Claim 35, wherein the ionic detergent is selected from the group consisting of cetyltrimethylammonium bromide, sodium dodecyl sulfate, sodium deoxycholate, 3-[(3-colamidopropyl)dimethylamino]-1-propane sulfonic acid, hexadecyltrimethylammonium bromide and myristylsulfobetaine.

54. The method of Claim 46, wherein the ionic detergent is selected from the group consisting of cetyltrimethylammonium bromide, sodium dodecyl sulfate, sodium deoxycholate, 3-[(3-colamidopropyl)dimethylamino]-1-propane sulfonic acid, hexadecyltrimethylammonium bromide and myristylsulfobetaine.